VERTICAL MIGRATION RHYTHMS OF NEWLY HATCHED LARVAE OF THE ESTUARINE CRAB, *RHITHROPANOPEUS HARRISII*

THOMAS W. CRONIN[1] AND RICHARD B. FORWARD, JR.

Duke University Marine Laboratory, Beaufort, NC 28516 and Department of Zoology, Duke University, Durham, NC 27706

ABSTRACT

Zoea larvae of the estuarine crab *Rhithropanopeus harrisii* were maintained in constant conditions in the laboratory, and their vertical migrations were followed for two or more days. Larvae which hatched in the laboratory, but which underwent embryonic development in an estuary having semidiurnal tides, often expressed circatidal rhythms in vertical migration. However, first-stage zoea larvae collected by plankton net in the same estuary had circatidal vertical migration rhythms of much greater amplitude and with a constant phase with respect to the natural tidal cycle. Laboratory-hatched larvae of crabs from an estuary with aperiodic tides had more variable vertical migrations, and field-caught larvae from the same habitat never expressed clear migration rhythms. When reared to the third zoeal stage in the laboratory under a diel light:dark cycle, larvae from both estuaries usually migrated arhythmically under constant conditions. Vertical migration rhythms of larvae of this species appear to be strongly predisposed to entrainment by natural tidal cues. Such migrations probably contribute to estuarine retention of the developing larvae.

INTRODUCTION

Estuaries are characterized by rapidly changing environmental conditions which often stress the organisms inhabiting them. In spite of this, larvae of the estuarine crab *Rhithropanopeus harrisii* are capable of remaining within an estuary near parent crab populations throughout development (Sandifer, 1973, 1975; Cronin, 1982). Retention is assisted by means of vertical migrations between the landward-flowing and seaward-flowing layers of the estuary; these migrations are partly under endogenous control. Cronin and Forward (1979) showed that *R. harrisii* larvae from an estuary with strong semidiurnal tides continued tidal vertical migrations in constant laboratory conditions, whereas laboratory-reared larvae from an estuary with irregular tides expressed a weak circadian rhythm. The tidal vertical migration was probably due to a circatidal rhythm in activity (Forward and Cronin, 1980).

R. harrisii passes through 4 zoeal stages before molting to the postlarva (Connolly, 1925). In our previous study of rhythmicity in vertical migration, experiments began with the stage III zoea, which were collected directly from the plankton and thus had had several days in which to become entrained to the estuarine tidal cycle. Yet the first-stage larvae of this species also migrate vertically under natural tidal conditions (Cronin, 1982). We therefore initiated a series of experiments to learn

whether newly hatched larvae also possess vertical migration rhythms. Migrations of larvae which hatched in the laboratory from crabs collected just before larval release occurred were compared to those of first stage larvae collected in the field. To understand better the origins of rhythmic behavior, we compared larvae from a population of crabs living in an estuary having semidiurnal tides with those from an estuary with irregular tides. Finally, the behavior of these newly hatched larvae was compared with that of later-stage larvae entrained under laboratory or field conditions. We found that newly hatched larvae could perform rhythmic migrations, but that the particular pattern of the migration varied with larval age and habitat.

MATERIALS AND METHODS

Preparation of larvae

Larvae from the estuarine crab *Rhithropanopeus harrisii* (Gould) were used exclusively in these experiments. Larvae were obtained from two populations of crabs in North Carolina, one population occurring in the Newport River estuary and the other in the Neuse River estuary. Conditions in these two estuarine systems differ strikingly (Forward *et al.*, 1982). The Newport River is strongly tidal with equal semidiurnal tides. It has extremely dark-colored water in its upper reaches (Cronin, 1982), and light intensities on the bottom, where *R. harrisii* adults live, are below the crabs' threshold (Forward *et al.*, 1982). The Neuse River has aperiodic tides (Roelofs and Bumpus, 1953) and contains rather transparent water.

Field-caught larvae were taken at each site by towing plankton nets within 1 m of the water's surface. These larvae were taken directly to the laboratory where the desired stage(s) of zoea larvae were identified using a dissecting microscope and placed into newly prepared water of identical salinity to that in which they were collected. Water of desired salinity was made by mixing filtered sea water with distilled water. In the Newport River, larvae were collected at high tide during the daytime by towing the sampling net from an outboard motor boat. Neuse River larvae were caught in plankton nets as we walked in shallow water at night. Temperatures at both collecting sites were between 25° and 30°C.

Laboratory-hatched larvae were obtained from recently collected crabs. Crabs were maintained in salinities similar to those of the collection site, in constant temperature (28 ± 1°C) and low-level light. These conditions are identical to those previously used for crabs originally entrained in natural environments (Forward *et al.*, 1982). In order that prior possible entrainment of larval rhythms be altered as little as possible, only hatches occurring within 4 days of collection were used. In the majority of cases (11 of 15 laboratory hatches), larvae hatched within 24 hours of collection of the mother crab. In a few experiments, larvae were reared to the third stage zoea before experiments were begun. These larvae were changed daily to clean water of appropriate salinity and fed newly hatched *Artemia salina* nauplii until the day of the molt to stage III.

In all cases, larvae were placed in clean water of identical salinity to that of previous field or laboratory exposure and allowed to feed on newly hatched *Artemia* nauplii for at least ½ hour before experiments began. Larvae were then transferred once more to clean water, and the desired number of individuals (usually 100, but occasionally fewer for field-caught larvae; see Table I) were added to a vertical lucite column. The dimensions of the enclosed water column were 190 cm tall × 5.0 cm × 7.5 cm.

TABLE I

Basic information about vertical migration experiments

Experiment Number	Starting date	Larval source estuary	Hatch location	Larval stage at start	Length of experiment (h)	Initial number of larvae
791	Aug 13, 1979	Newport	Laboratory	I	83	100
792	Aug 17, 1979	Newport	Laboratory	I	70	100
793	Aug 29, 1979	Newport	Field	I	77	100
794	Sept 7, 1979	Neuse	Laboratory	I	49	100
801	Aug 7, 1980	Neuse	Laboratory	I	52	100
802	Aug 13, 1980	Newport	Field	I	42	100
803	Aug 15, 1980	Neuse	Laboratory	I	69	100
804	Aug 22, 1980	Neuse	Laboratory	I	67	100
805	Aug 27, 1980	Newport	Laboratory	I	54	100
811	Aug 11, 1981	Newport	Laboratory	I	77	100
812	Aug 14, 1981	Neuse	Laboratory	I	71	100
813	Aug 19, 1981	Newport	Laboratory	I	62	100
814	Aug 26, 1981	Neuse	Field	I	54	100
815	Aug 31, 1981	Neuse	Field	I	45	100
816	Sept 3, 1981	Neuse	Field	I	46	100
817	Sept 10, 1981	Neuse	Field	I	51	100
821	July 7, 1982	Neuse	Field	IV	51	37
822	July 10, 1982	Neuse	Field	IV	56	24
823	July 15, 1982	Neuse	Field	III & IV	77	80
824	July 18, 1982	Neuse	Laboratory	III	83	100
825	July 22, 1982	Neuse	Field	III & IV	76	100
826	Aug 10, 1982	Neuse	Laboratory	III	105	100
827	July 26, 1982	Newport	Laboratory	III	97	100
828	Aug 4, 1982	Newport	Laboratory	III	108	100
829	Aug 31, 1982	Newport	Laboratory	III	102	100

Monitoring of larval vertical distributions

Once placed in the experimental column, larvae were maintained in constant darkness and temperature (experiments 791 and 792, 21 ± 1°C; all others, 25 ± 1°C). Because larvae were not fed again, the total length of each experiment was limited by the ability of each larval population to resist starvation. Experiments were usually permitted to run until larval mortality and deterioration left fewer than 20% of the original number of larvae in the water column; occasionally, experiments were terminated before this point if greater than 50 h of data had been obtained.

Distributions of larvae were determined by the method of Cronin and Forward (1979), the only difference being that the experimental column was backlit with diffuse infrared light passing through a Kodak Wratten 87 filter (50% transmission wavelength, 790 nm). Briefly, a closed-circuit TV camera equipped with a silicon-target vidicon vertically scanned the entire height of the lucite column once each half hour, and the camera's output was stored on videotape for later analysis. The infrared backlight was switched on only during the 2 min scan time; the camera also passed a clock on each scan to record the time of day.

Videotapes were analyzed during replay by counting the number of larvae in each 10 cm segment of the water column. Larvae actually on the bottom were not counted, since our experience has been that over 80 percent of well-fed larvae remain

in the water column. The two counts obtained each hour for each segment were summed and an hourly depth-weighted mean calculated. All analyses were performed on the time series of mean depth values.

Data analysis

These experiments were designed to reveal rhythmic behavior in crab larvae following entrainment in specific embryonic or early larval environments. The resulting data challenged straightforward time series analysis for several reasons (see the Figures). Records were short in length, usually less than 72 h. Data represented output from groups of larvae whose individuals did not necessarily have highly synchronous behavior. There commonly were long-term vertical trends. Larval vertical migrations were often of low amplitude and included considerable noise. Of benefit to data analysis, however, was the fact that we restricted our interest to rhythms of circatidal or circadian periods, since these were the periods observed in the earlier study (Cronin and Forward, 1979). Following consultation with J. Hartigan, Yale University Department of Statistics, we decided to analyze each data set using three statistical techniques and a visual evaluation of the data. Each statistical method of analysis approached the data from an independent point of view, and it was common for one method to indicate rhythmicity where the others did not. All analytical methods required complete time series. In the two cases in which some data were missing due to equipment failure, missing values were replaced with values calculated by linear interpolation between adjacent measured values. Because the use of the statistical analyses was useful for extracting information about larval rhythms from the raw data, they are described in some detail below.

Fisher's Periodogram Test. To perform a rough linear detrend, a regression line was fitted to the raw data and subtracted from all points. Detrended data were subjected to Fisher's periodogram test (Fuller, 1976). This test only applies to harmonics of the total time series; periods of significant cycles can be compared to the tidal or diel period, but because the tested cycles are harmonics of the series they do not always fall very near the precise environmental period. Fisher's test has the further limitation of examining only the frequency of largest amplitude in a time series.

Multiple Autoregression. Each data set was multiply regressed on itself at three lags. For the circatidal rhythm analysis, the 3 independent variables were the measured mean depth values at 1 h, 2 h, and 12 h or 13 h prior to the value at a given hour. (Both 12 h and 13 h were tested in order to bracket the average natural tidal period, which was 12.4 h in the Newport River.) For circadian rhythm analysis, the lags were 1 h, 2 h, and 24 h. Periodicity in the data was taken to be significant if the regression coefficient of the 3rd independent variable (lag of 12, 13, or 24 h) was significantly greater than 0. Multiple autoregression was relatively inefficient in finding rhythms in these experiments.

Analysis of Variance (ANOVA). ANOVA is not a traditional statistical tool for time series analysis. We were able to apply it because we restricted our analytical effort to periods approximating the natural diel and tidal cycles. Each data set was broken into a whole number of segments; for tidal analysis these segments were 12 h or 13 h in length, while for diel analysis they were 24 h long. To minimize the effects of long-term trends, the mean value in each segment was calculated and removed from all values in that segment. Next, a 1-way ANOVA was performed on the 12, 13, or 24 hourly values, with the number of replicates in each hour being the total number of segments in the data set. This analysis therefore tested whether

there was significant hour-to-hour variation in the data within blocks 12 h, 13 h, or 24 h in length. A significant result could occur when the averaged segments contained a monotonic trend, when high-frequency noise was present, when repeated smaller cycles fell within the total segment length, or when cycles of the total segment length occured. Since only the last case was of interest, the sequence of hourly means was examined for rejection of misleading significance due to trends, noise, or internal cycles. ANOVA proved to be a powerful method for determining rhythms in our data, probably because the method of removing the mean from each section of data was an effective way to minimize the contributions of irregular long-term variations.

Subjective Evaluation of Data. Because of the nature of the process under study, it is probably at present impossible to obtain data which are completely amenable to statistical treatment. We have relied on the techniques described above to provide an objective base for drawing conclusions, but occasionally we also turned to a subjective evaluation of the data in hopes of increasing our understanding of larval rhythmic behavior. We encourage readers to inspect thoroughly the data we present so that they can decide whether to accept our conclusions.

RESULTS

Essential information about each experiment is given in Table I. When classed according to larval source (Newport vs. Neuse River), hatch location (laboratory vs. field), and larval stage at the beginning of the experiment (zoea I vs. zoea III or IV), a total of 7 types of experiments was performed. [Ideally, there should have been 8 possible combinations of categories, but results obtained with late-stage larvae collected in the Newport River have been reported earlier (Cronin and Forward, 1979)]. Experiments are grouped by type in Figures 1–7, and results of statistical analyses are given in Table II. For convenience in presenting results, each type of experiment will be described separately.

Newport River: Laboratory-hatched stage I zoea larvae

This series of experiments investigated rhythmic vertical migration behavior in newly hatched larvae which had been entrained as embryos in the strongly tidal conditions of the Newport River estuary, but which hatched in the laboratory. Five replicates were performed (Fig. 1), and of these, 4 revealed significant evidence of circatidal rhythmicity in larval vertical migration (Table II). The 5th experiment also illustrated circatidal periodicity after an initial 24 h rise in the water column (Table II, Fig. 1). Visual examination of Figure 1 reveals that low points in the larval migration were not particularly well synchronized with the time of low tide at the site of collection of the parent crab. No evidence was found in any analysis for circadian rhythmicity, nor is any circadian variation suggested in the individual graphs of Figure 1. Experiment 813 is a possible exception, since alternately deeper low points occurred near midnight (Fig. 1).

Neuse River: Laboratory-hatched stage I zoea larvae

These experiments were similar to those of the previous group except that prior entrainment occurred in the nontidal, well lit environment of the Neuse River estuary. All larvae hatched near the time of sunset on the night the experiment began, as is typical of larval hatches of crabs from this location (Forward *et al.*, 1982). The results were more varied than those obtained with Newport River larvae (Fig. 2). Data analysis revealed significant circatidal periodicity in 2 cases (experi-

TABLE II

Results of statistical tests for periodicity performed on the time series of each experiment's data*

Experiment type	Experiment number	Fisher's test	Multiple autoregression	Analysis of variance
Newport River, First	791	+	+	+
Stage, Lab-Hatched	792	11.7 h	—	12 h, 13 h
	805	—	—	12 h
	811	—	—	12 h
	813	—	13 h	—
Neuse River, First Stage,	794	—	—	—
Lab-Hatched	801	13.0 h	—	12 h, 13 h
	803	34.5 h	—	—
	804	—	—	24 h
	812	—	—	12 h, 24 h
Newport River, First	793	12.8 h	12 h, 24 h	12 h, 13 h
Stage, Field-Caught	802	14.0 h	12 h, 13 h, 24 h	12 h, 13 h
Neuse River, First Stage,	814	—	—	—
Field-Caught	815	—	—	—
	816	—	—	—
	817	—	—	—
Newport River, Late	827	—	—	—
Stage, Lab-Hatched	828	—	—	12 h, 13 h
	829	—	—	—
Neuse River, Late Stage,	824	—	—	—
Lab-Hatched	826	—	—	—
Neuse River, Late Stage,	821	25.5 h	—	—
Field-Caught	822	18.7 h	—	—
	823	—	—	—
	825	—	—	—

* Included are all results of statistical tests giving $P < 0.05$. Significant results are given for Fisher's Periodogram Test only if the significant period was not equal to the entire length of the time series.

+ In experiment 791, no test yielded a significant result for the entire 83 h of the time series. However, if the first 24 h of data (during which there was a continuous rise) were eliminated, Fisher's Test indicated a significant period of 11.8 h, and ANOVA gave significant results for periods of 12 h and 13 h.

ments 801 and 812, Table II); circadian periodicity was indicated in 2 experiments as well (804 and 812). In spite of the heterogeneous mixture of vertical migration patterns, one migration feature was consistently observed. All groups of larvae initially migrated downward until near midnight (near dawn in experiment 794), when they reversed their course and rose for the succeeding several hours (Fig. 2). This pattern is not simply a response to being placed in the experimental column, since it was not observed in Newport River larvae (Fig. 1). Subsequent pre-dawn rises are also visible in several cases (Experiments 801, 803, 804, and 812).

Newport River: Field-caught stage I zoea larvae

In these experiments, larvae were taken from the plankton and thus had an opportunity to experience conditions in the Newport River as free-living individuals for some time prior to being placed under constant conditions. Only two experiments were performed since the results were very clearcut. Larvae were strongly circatidally rhythmic (Table II), reaching the low points of their migrations just after the time
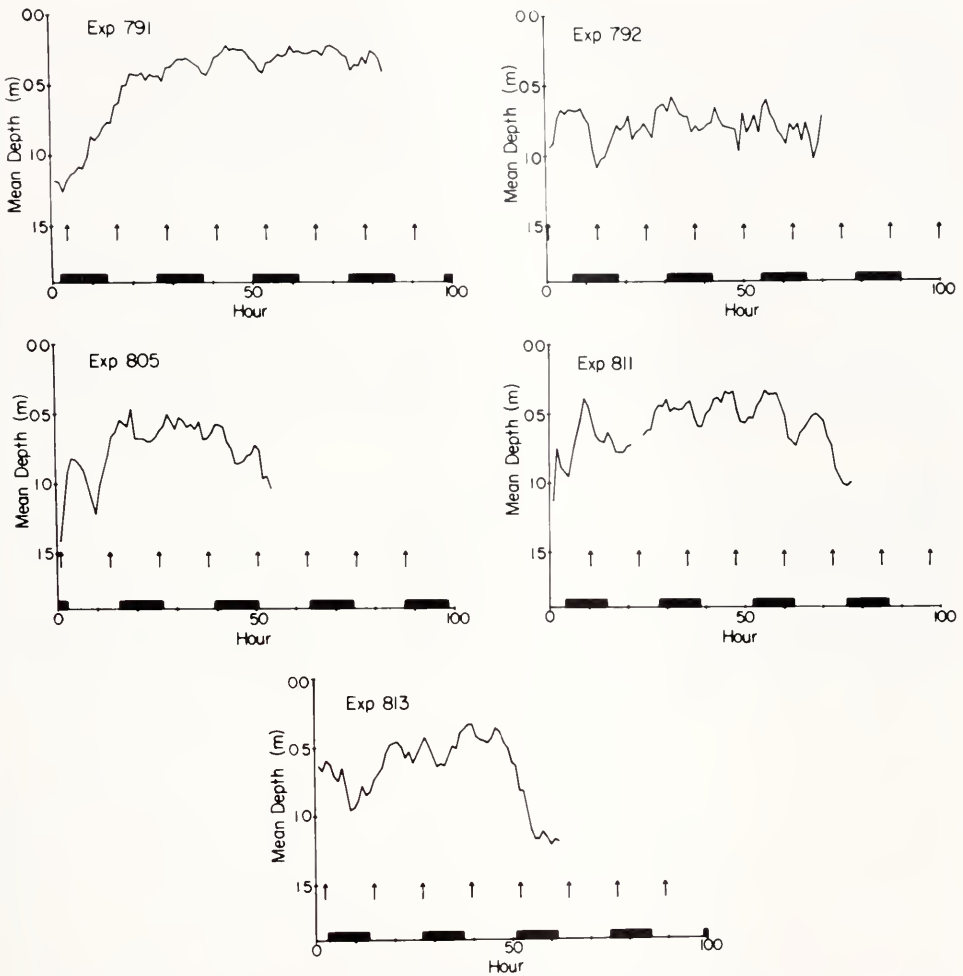
FIGURE 1. Hourly positions of the mean depths of populations of first-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with larvae of a single hatch from a crab collected in the Newport River estuary. Dark and light bands on the abscissa indicate the times of natural night and day, respectively. Arrows indicate times of low tide at the collection site. Gaps correspond to missing data due to equipment failure.

of low tide at the collection site (Fig. 3). Such a pattern duplicates results already obtained for field-entrained late-stage Newport River *R. harrisii* larvae (Cronin and Forward, 1979). Autoregression analysis also showed that larval depths were significantly predicted by those 24 h earlier in the record (Table II), but we feel that this is actually a correlation with the second previous tidal cycle. The other statistical tests found no 24 h rhythmicity, and inspection of Figure 3 shows no sign of circadian activity.

Neuse River: Field-caught stage I zoea larvae

Here, larval sample populations were taken in nightime plankton tows in the Neuse River. The majority of the larvae almost certainly hatched on the same night
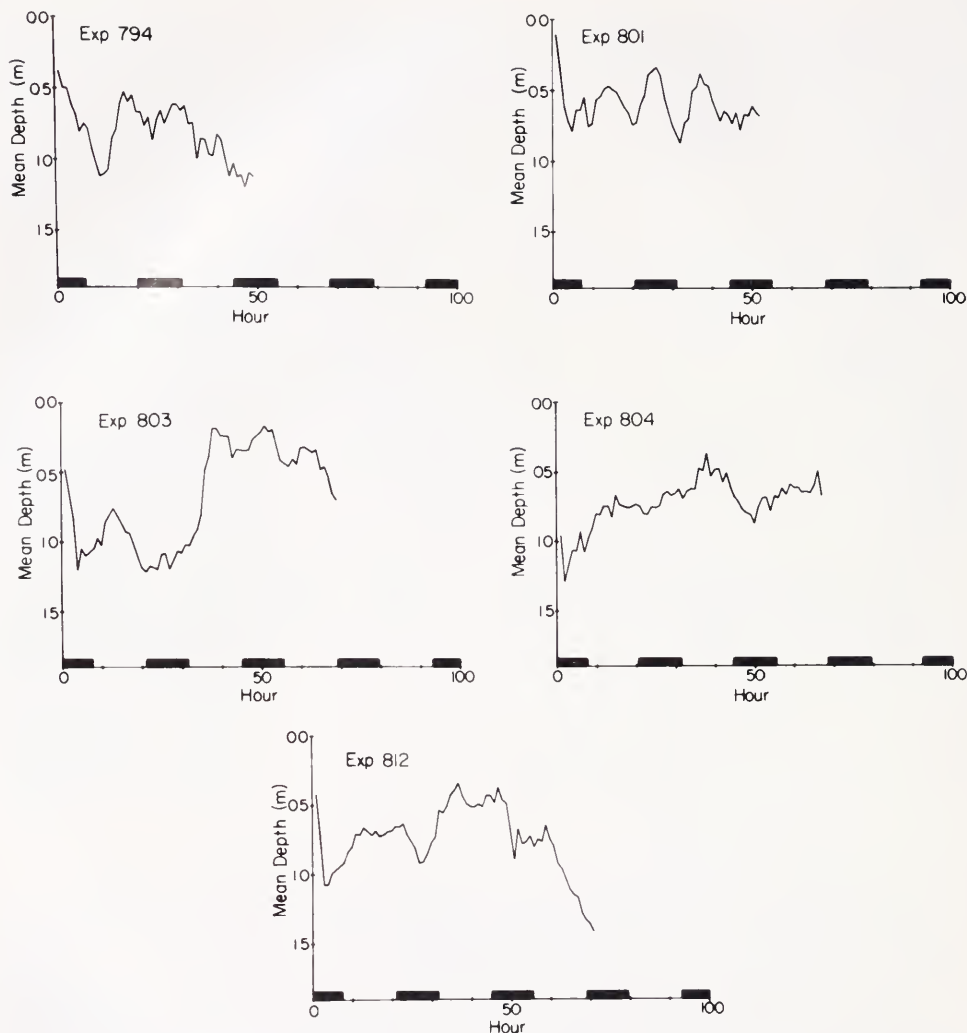
FIGURE 2. Hourly positions of the mean depths of populations of first-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with larvae of a single hatch from a crab collected in the Neuse River estuary. Dark and light bands on the abscissa indicate the times of natural night and day, respectively.

on which they were collected (see Discussion). Four replicates were completed. Results revealed a variety of irregular migration patterns (Fig. 4), but in no case was there any significant circadian or circatidal rhythmicity (Table II). However, the pattern described earlier for laboratory-hatched Neuse River larvae was again present. Larvae usually moved downward until late in the first dark phase, at which time they reversed course and rose in the water column. In these experiments, however, there was little evidence for repeated cycling (Fig. 4).

Newport River: Laboratory-hatched and reared late-stage zoea larvae

We have previously reported that Neuse River larvae reared in constant temperature and in a 12 h light:12 h dark cycle performed a low-amplitude circadian
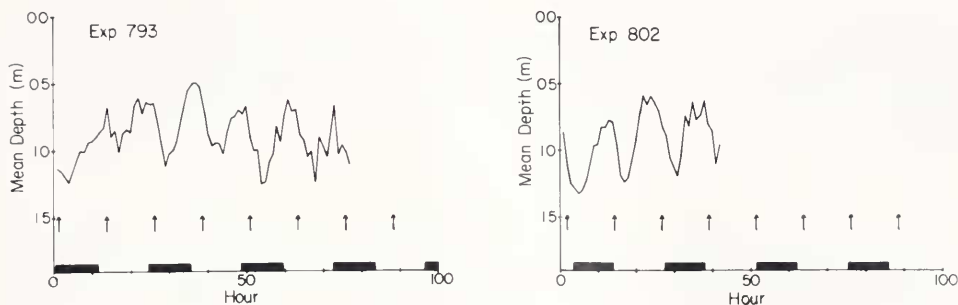
FIGURE 3. Hourly positions of the mean depths of populations of first-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with larvae caught in plankton net tows taken in the Newport River estuary. Dark and light bands on the abscissa indicate the times of natural night and day, respectively. Arrows indicate times of low tide at the collection site.

rhythm of vertical migration, reaching their lowest position near midnight and their greatest height near midday (Cronin and Forward, 1979). We were interested to learn whether the same result would obtain with laboratory-reared Newport River larvae; therefore, on 3 occasions we reared laboratory-hatched Newport River larvae to zoea III before placing them in experimental conditions. Larvae were maintained in a 14 h light:10 h dark cycle which closely matched the actual times of sunrise
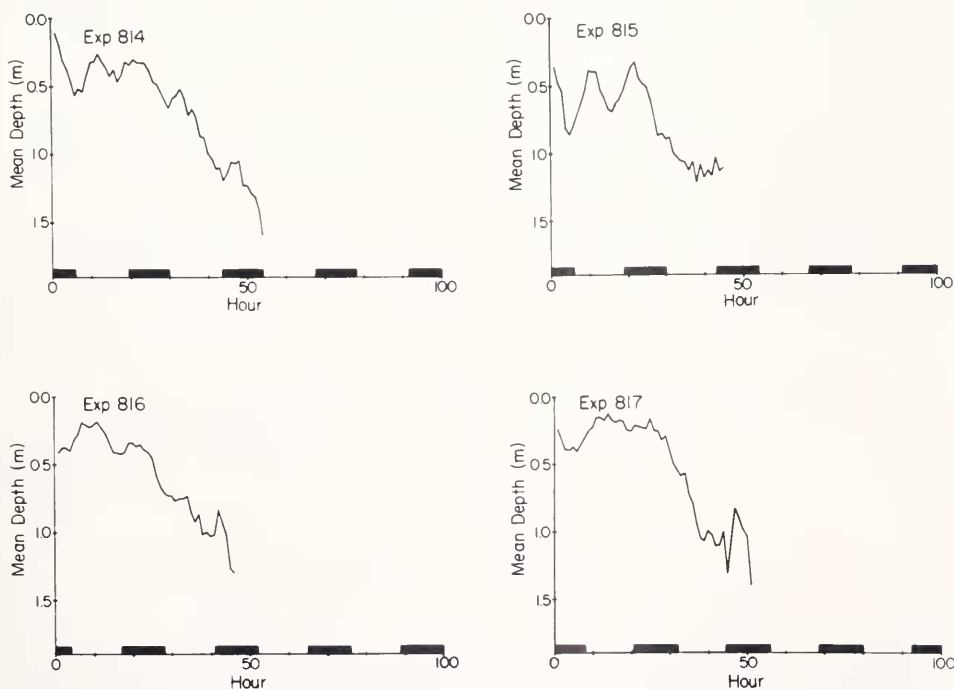

FIGURE 4. Hourly positions of the mean depths of populations of first-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with larvae caught in plankton net tows taken in the Neuse River estuary. Dark and light bands on the abscissa indicate the times of natural night and day, respectively.
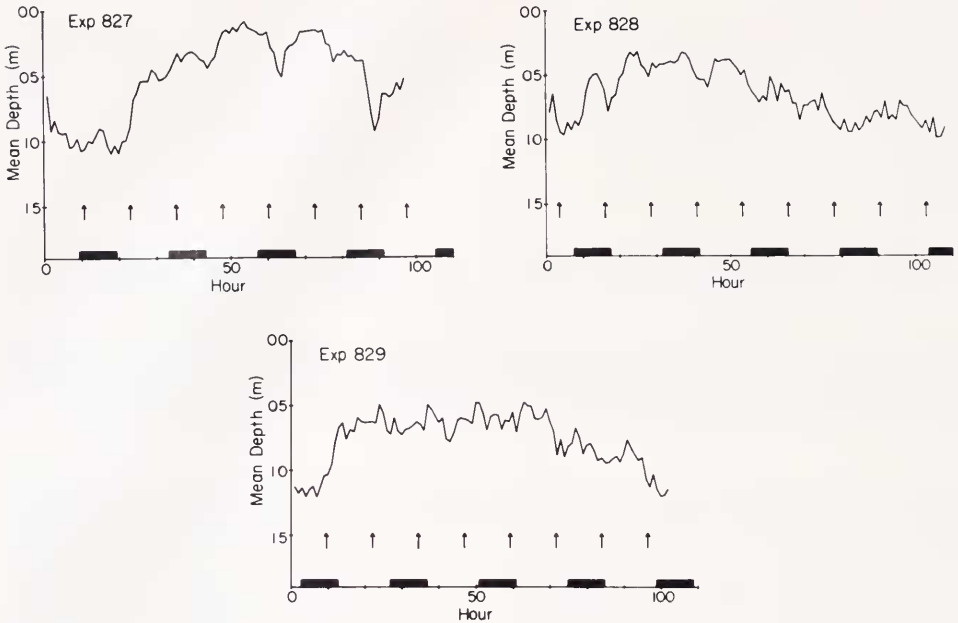
FIGURE 5. Hourly positions of the mean depths of populations of late-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with larvae of a single hatch from a crab collected in the Newport River estuary, which were reared to zoeal stage III before the experiment began. Light and dark bands on the abscissa indicate the light:dark cycle to which the larvae were exposed during rearing. Arrows indicate times of low tide at the collection site.

and sunset. Migration patterns were largely random (Fig. 5), but in one case (experiment 828) ANOVA indicated the presence of circatidal rhythmicity (Table II).

Neuse River: Laboratory-hatched and reared late stage zoea larvae

For these 2 experiments, Neuse River larvae were prepared under identical conditions to the Newport River larvae just described. Once more, the light:dark cycle
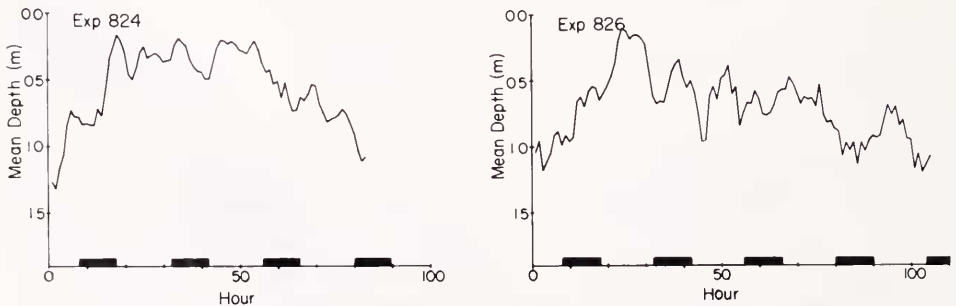

FIGURE 6. Hourly positions of the mean depths of populations of late-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with larvae of a single hatch from a crab collected in the Neuse River estuary, which were reared to zoeal stage III before the experiment began. Light and dark bands on the abscissa indicate the light:dark cycle to which the larvae were exposed during rearing.

consisted of 14 h of light alternating with 10 h of dark, instead of the 12 h:12 h cycle previously used (Cronin and Forward, 1979). In contrast to the results of that work, no rhythmicity was indicated, either statistically or by visual inspection of the data (Fig. 6, Table II). Essentially random movements of the center of the larval population occurred.

Neuse River: Field-caught late-stage zoea larvae

Larvae were collected in an identical way to the first-stage Neuse River larvae. Four replicates were performed (Fig. 7); there was some statistical evidence in two of them of circadian rhythmicity (experiments 821 and possibly 822, Table II). However, the form of the circadian pattern is difficult to recognize in the figures, and all the graphs reveal considerable random movement.

DISCUSSION

Larvae of the estuarine crab *Rhithropanopeus harrisii* are known to possess endogenous rhythms of vertical migration by the time they attain the third zoeal stage (Cronin and Forward, 1979). The period lengths of these rhythms can approximate the period of either the tidal cycle or the diel cycle, depending on the prior entrainment regime and the larval source. We designed the experiments described in this paper to answer the questions of whether these rhythms are expressed early in larval life and whether it is possible for larvae to become entrained to
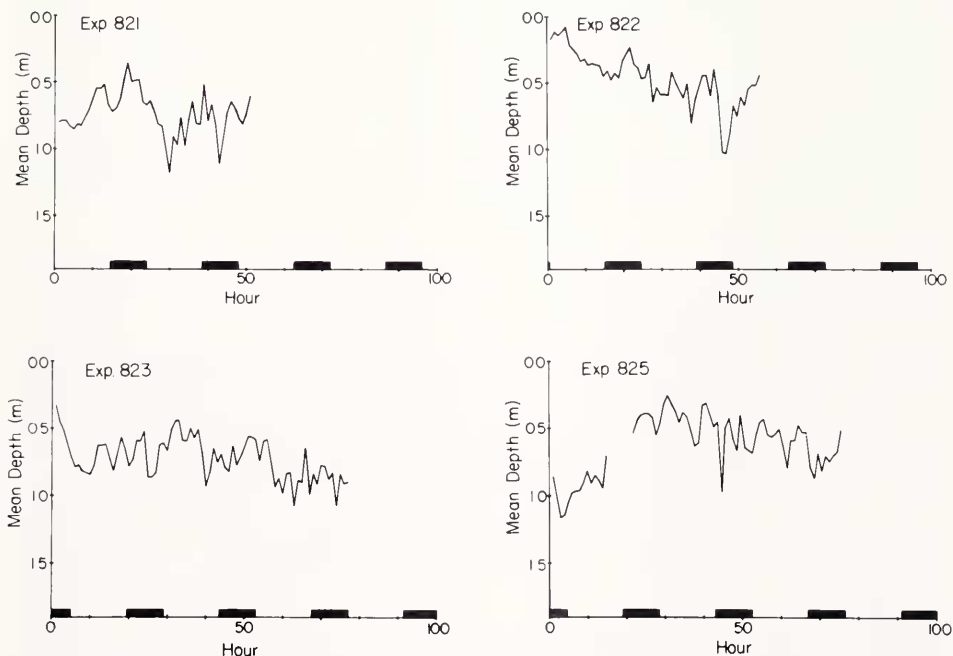

FIGURE 7. Hourly positions of the mean depths of populations of late-stage *Rhithropanopeus harrisii* larvae maintained in constant darkness and at constant temperature. Each panel represents the results obtained with stage III and/or stage IV larvae collected by plankton net in the Neuse River estuary. Dark and light bands on the abscissa indicate the times of natural night and day, respectively. Gaps correspond to missing data due to equipment failure.

environmental cycles during their embryonic development. The results, although more equivocal than the ones obtained previously, suggest that both questions may be answered in the affirmative. However, conditions which vary between estuaries, and which also differ in their effects on larvae before and after hatching, strongly modify the rhythmic aspects of larval migrations. Furthermore, there probably exist differences among larval cohorts or larval populations which also affect expression of larval rhythms.

Stage I zoea larvae from the Newport River performed vertical migrations in the constant conditions of the laboratory, even if they had never experienced the strong tidal influences of this estuary as free-living individuals. The apparent circatidal rhythms of laboratory-hatched larvae were probably entrained during embryonic development. While the larvae are developing, the parent crab with the egg mass remains at depths where the diel light:dark cycle is imperceptible (Forward *et al.,* 1982), so the lack of circadian rhythmicity is not surprising. The observed rhythmicity could be a product of embryonic entrainment by tidal cycles of pressure and/or salinity, as both are known to induce circatidal rhythmicity in crustaceans (Naylor and Atkinson, 1972; Taylor and Naylor, 1977). Pressure receptors have not been described in larval brachyurans, much less in their embryos; but *R. harrisii* larvae are highly responsive to pressure changes (Bentley and Sulkin, 1977; Wheeler and Epifanio, 1978). They also respond to small salinity changes (Latz and Forward, 1977; Harges and Forward, 1982).

The mother crab could assist in entraining larval rhythms by manipulating the egg masses at a specific phase in the tidal cycle, since mechanical stimulation is effective in entraining circatidal rhythmicity (Enright, 1963, 1965). We have never observed such behavior in crabs in the laboratory. Hatching itself could be a "one-shot" synchronizer since larval release is precisely timed with respect to environmental cycles in *R. harrisii* (Forward *et al.,* 1982) as well as in other estuarine crabs (DeCoursey, 1979; Bergin, 1981; Saigusa, 1981). This seems unlikely since crabs from the Neuse River have a larval release rhythm, but their larvae do not always reveal circatidal rhythms after hatching. Furthermore, unpublished observations suggest that the time of hatching is more likely controlled by the embryos themselves than by the mother crab.

In any case, much stronger rhythms are expressed by stage I larvae from the Newport River after a short time in the plankton. Our field-caught larvae were probably less than 3 days old, since the first zoeal stage is passed in 2–3 days at environmental temperatures in the laboratory (Costlow and Bookhout, 1971). In the few tidal cycles after hatching, the larval rhythms became enhanced in amplitude and, probably, coherency (seen as reduced noise, c.f. Fig. 1 and 3), and thus became very similar to rhythms of late-stage *R. harrisii* larvae from the same location (Cronin and Forward, 1979). The entraining stimuli, whether the same or different, are clearly much more effective upon free-living larvae than on developing embryos.

Compared to Newport River larvae, stage I zoea larvae from the Neuse River were much more variable in their expression of vertical migration rhythms. Two experiments showed statistical evidence of circadian rhythmicity and 2 of circatidal rhythmicity, while one group of laboratory-hatched larvae and all field-caught larval groups were arhythmic. The statistical results agree reasonably well with the subjective appearance of the results (Fig. 4). Circadian rhythmicity is not unexpected in this population since hatching time is under circadian rhythmic control (Forward *et al.,* 1982) and late-stage larvae of crabs from this estuary can show circadian rhythms of vertical movement (Cronin and Forward, 1979). The crabs were collected from shallow depths in quite transparent water, so entrainment by the daily

light:dark cycle was possible. In all these experiments there was an initial nightime sinking phase followed by an upward migration early the next day (experiment 816 did not have the initial descent, but this experiment began just before dawn). The results thus resemble the single-cycle "hourglass" timing of the vertical migrations of some marine zooplankton, which require resetting by external inputs each day (Enright and Hamner, 1967). It appears that newly-hatched larvae from the Neuse River can express a weak, rapidly-damping circadian rhythm in vertical migration.

Evidence that at least some cohorts of Neuse River larvae have circatidal rhythms is surprising. Tides in this estuary are reportedly aperiodic (Roelofs and Bumpus, 1953). We measured changes in salinity and depth at one of our collection sites in the Neuse River for 24 h and found no evidence of regular tidal influence. However, *R. harrisii* from this estuary have circatidal hatching rhythms once placed in natural tidal conditions (Forward *et al.*, 1982). Until further experiments are done, it will be impossible to know whether the 12–13 h rhythms observed here are expressions of this innate tidal clock.

Only laboratory hatches of Neuse River larvae had significant rhythms. This probably reflects the fact that in each experiment, larvae had identical developmental histories and a single hatching time. In the field samples, first-stage *R. harrisii* larvae were much more abundant than later stages, and were most easily obtained soon after dark. Therefore, the ones used in our experiments had most likely hatched on the night they were collected, but they had experienced a range of developmental conditions and probably had hatched over a period of hours (see Forward *et al.*, 1982). The irregular vertical movements of these larval groups evidently result from the lack of synchrony among the individual larvae of the experiment. This contrasts with the greater synchrony seen in the migrations of Newport River larvae entrained in the field. Evidently, tidal variables of latter estuary are much more effective synchronizers than the diel variations in the Neuse River environment.

In their essential features, the migration patterns of laboratory-hatched stage I zoea larvae from each estuary were similar to those of first-stage larvae collected from the plankton of that estuary. In contrast, late-stage larvae reared in the laboratory had vertical migrations which resembled neither those of field-caught larvae of similar age nor those of the first-stage larvae. Newport River larvae which lived in natural field conditions until the third zoea had dramatic migration rhythms when placed in constant conditions (Cronin and Forward, 1979). When reared to the same stage in the laboratory in an imposed light:dark cycle, these larvae were usually arhythmic (Fig. 5). In one case, statistical analysis indicated that a cycle of circatidal period was present (Table II). It therefore remains possible that an initial circatidal rhythm can continue throughout development, though it is not clear how coherency among larval individuals could be maintained.

In neither laboratory-reared nor field-caught groups of stage III Neuse River larvae do vertical migration patterns commonly show significant rhythmicity. To the eye, these migrations appear essentially random. Larvae from this source are capable of expressing circadian migration rhythms when reared on a 12 h:12 h light:dark cycle (Cronin and Forward, 1979). The present results suggest that the circadian tendency is rather weak.

Taken as a whole, the results of this study indicate that entrainment of vertical migration rhythms may occur during embryonic development. Larvae of *R. harrisii* seem strongly biased towards circatidal, rather than circadian, rhythmicity. Entrainment to the tidal cycle is dramatically enhanced once larvae become free living, whereas entrainment to the diel cycle is clearly no more effective on free-living than on embryonic larvae. Our failure to find strong circadian rhythms after rearing

larvae in an imposed light:dark cycle is especially impressive when compared to results of a previous experiment which studied vertical migration of late-stage *R. harrisii* larvae when exposed to an external light:dark cycle (Cronin and Forward, 1982). In this case, exogenously driven diel vertical migrations occurred over most of the 1.9 m height of the experimental column. Such migrations have not been observed in field populations of these larvae (Cronin, 1982), and the importance of the diel light:dark cycle in controlling larval behavior of *R. harrisii* remains to be understood.

Several other species of estuarine crabs have circatidal rhythms of larval release (DeCoursey, 1979; Bergin, 1981; Saigusa, 1981), and field sampling by Christy and Stancyk (1982) strongly suggested that virtually all crab species in a South Carolina estuary release larvae near local high tide times. One might therefore expect circatidal rhythms in the larvae of these crabs. Yet *R. harrisii* stands alone among species yet studied in having highly effective mechanisms for larval retention in estuaries (Cronin, 1982); the other larval species all apparently undergo rapid export from estuaries, perhaps to reduce predation pressure (Christy, 1982). Estuarine retention of *R. harrisii* larvae is thought to be assisted by their tidal vertical migrations (Cronin and Forward, 1979, 1982; Cronin, 1982). Studies of the circatidal rhythms of other species of estuarine crab larvae, as well as of the endogenous and exogenous controls on these rhythms, should prove highly informative to our understanding of the bases of larval ecology.

LITERATURE CITED

BENTLEY, E., AND S. D. SULKIN. 1977. The ontogeny of barokinesis during zoeal development of the xanthid crab *Rhithropanopeus harrisii* (Gould). *Mar. Behav. Physiol.* **4**: 275–282.

BERGIN, M. E. 1981. Hatching rhythms in *Uca pugilator* (Decapoda: Brachyura). *Mar. Biol.* **63**: 151–158.

CHRISTY, J. H. 1982. Adaptive significance of semilunar cycles of larval release in fiddler crabs (genus *Uca*): test of an hypothesis. *Biol. Bull.* **163**: 251–263.

CHRISTY, J. H., AND S. E. STANCYK. 1982. Timing of larval production and flux of invertebrate larvae in a well-mixed estuary. Pp. 489–503 in *Estuarine Comparisons,* V. S. Kennedy, ed. Academic Press, New York.

CONNOLLY, C. J. 1925. The larval stages and megalopa of *Rhithropanopeus harrisii* (Gould). *Contr. Can. Biol.* **2**: 329–334.

COSTLOW, J. D., JR., AND C. G. BOOKHOUT. 1971. The effect of cyclic temperatures on larval development in the mud-crab *Rhithropanopeus harrisii.* Pp. 211–220 in *Fourth European Marine Biology Symposium,* D. J. Crisp, ed. Cambridge Univ. Press, Cambridge.

CRONIN, T. W. 1982. Estuarine retention of larvae of the crab *Rhithropanopeus harrisii. Estuarine Coastal Shelf Sci.* **15**: 207–220.

CRONIN, T. W., AND R. B. FORWARD, JR. 1979. Tidal vertical migration: an endogenous rhythm in estuarine crab larvae. *Science* **205**: 1020–1022.

CRONIN, T. W., AND R. B. FORWARD, JR. 1982. Tidally timed behavior: effects on larval distributions in estuaries. Pp. 505–520 in *Estuarine Comparisons,* V. Kennedy, ed. Academic Press, New York.

DECOURSEY, P. 1979. Egg hatching rhythms in three species of fiddler crabs. Pp. 399–406 in *Cyclic Phenomena in Marine Plants and Animals,* E. Naylor and R. G. Hartnoll, eds. Proceedings of the 13th European Marine Biology Symposium, Pergamon Press, Oxford.

ENRIGHT, J. T. 1963. The tidal rhythm of activity of a sand-beach amphipod. *Z. Vgl. Physiol.* **46**: 276–313.

ENRIGHT, J. T. 1965. Entrainment of a tidal rhythm. *Science* **147**: 864–867.

ENRIGHT, J. T., AND W. M. HAMMER. 1967. Vertical diurnal migration and endogenous rhythmicity. *Science* **157**: 937–941.

FORWARD, R. B., JR., AND T. W. CRONIN. 1980. Tidal rhythms of activity and phototaxis in an estuarine crab larva. *Biol. Bull.* **163**: 295–303.

FORWARD, R. B., JR., K. LOHMANN, AND T. W. CRONIN. 1982. Rhythms in larval release by an estuarine crab *(Rhithropanopeus harrisii)*. *Biol. Bull.* **163**: 287–300.

FULLER, W. A. 1976. *Introduction to Statistical Time Series.* J. Wiley, New York. 470 pp.

HARGES, P., AND R. B. FORWARD, JR. 1982. Salinity perception by larvae of the crab *Rhithropanopeus harrisii* (Gould). *Mar. Behav. Physiol.* **8**: 311–331.

LATZ, M. I., AND R. B. FORWARD, JR. 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. *Biol. Bull.* **153**: 163–179.

NAYLOR, E., AND R. J. A. ATKINSON. 1972. Pressure and the rhythmic behaviour of inshore marine animals. *Symp. Soc. Exp. Biol.* **26**: 395–415.

ROELOFS, E. W., AND D. F. BUMPUS. 1953. The hydrography of Pamlico Sound. *Bull. Mar. Sci. Gulf Carib.* **3**: 181–205.

SAIGUSA, M. 1981. Adaptive significance of a semilunar rhythm in the terrestrial crab *Sesarma*. *Biol. Bull.* **160**: 311–321.

SANDIFER, P. A. 1973. Distribution and abundance of decapod crustacean larvae in the York River estuary and adjacent lower Chesapeake Bay, Virginia, 1968–1969. *Chesapeake Sci.* **14**: 235–257.

SANDIFER, P. A. 1975. The role of pelagic larvae in recruitment to populations of adult decapod crustaceans in the York River estuary and adjacent lower Chesapeake Bay, Virginia. *Estuarine Coastal Mar. Sci.* **3**: 269–279.

TAYLOR, A. C., AND E. NAYLOR. 1977. Entrainment of the locomotor rhythm of *Carcinus* by cycles of salinity change. *J. Mar. Biol. Assoc. U.K.* **57**: 273–277.

WHEELER, D. E., AND C. E. EPIFANIO. 1978. Behavioral response to hydrostatic pressure in larvae of two species of xanthid crabs. *Mar. Biol.* **46**: 167–174.